Ultrastructure of Glomerulopathy in Swine

Kinji SHIROTA, and Yasuo NOMURA

Department of Veterinary Pathology, School of Veterinary Medicine, Azabu University, Sagamihara 229, Japan

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ABSTRACT. Ultrastructural changes of the glomeruli were examined in the kidneys without gross lesions from 32 slaughtered pigs aged about six months. Of these, 24 pigs showed diffuse global mesangial enlargement with definite IgG and C3 deposition, and other eight had slight or no change in the glomeruli with little or no IgG or C3 deposition. The major ultrastructural abnormality was the electron-dense deposits in subendothelial, subepithelial and most frequently mesangial areas. Of the deposits in the mesangium, paramesangial ones were most characteristic. The degree of the deposition of electron-dense deposits generally coincided with that in light and immunofluorescence microscopy. In 20 of the 32 pigs, the clusters of spherical microparticles (SMP) were present in the mesangial, intramembranous, subendothelial and most frequently subepithelial areas. The incidence of SMP did not always coincide with the intensity in light microscopy, the immunofluorescent antibody test or the deposition of dense deposits. No conspicuous lesions of the glomerular basement membrane were observed except the electron-dense deposits and SMP.—**KEY WORDS:** dense deposit, glomerulonephropathy, renal pathology, swine, spherical microparticle.

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In a previous report [17], we revealed a high incidence of glomerulopathy in the swine kidneys having no gross lesions. The most common lesion was mesangial enlargement due to hypercellularity, increased mesangial matrix and hyaline deposits. IgG and third complement component (C3) deposits were found to be in concurrence with hyaline materials by immunofluorescent study.

From the study, we suspected that glomerulopathy in swine is analogous to IgA nephropathy in man [4]. Ultrastructural studies on IgA nephropathy revealed various abnormalities of glomerular basement membrane (GBM) in addition to mesangial lesions [6, 7, 14, 15, 19, 20]. This study was made to see the ultrastructural characteristics of swine glomerulopathy and to compare its morphological changes with those of IgA nephropathy in man.

MATERIALS AND METHODS

Kidneys without gross lesions from 32 of the 100 pigs reported previously [17] were examined. They were fixed in neutral phosphate-buffered 10% formalin and embedded in paraffin. Sections, 3- μ m thick, were stained with hematoxylin and eosin (HE), periodic acid-Schiff reaction (PAS), periodic acid-silver methenamine (PASM) or acid-fuchsin orange-G stain (AFOG) [13].

Small pieces of fresh kidneys were frozen in hexane-dry ice acetone. The frozen sections of 4- μ m were used for detection of IgG and C3 in the renal glomeruli by the immunofluorescent antibody test [17].

For electron microscopy, small renal samples were fixed in 2.5% glutaraldehyde for 2 hr, postfixed in 1% osmium tetroxide for 2 hr, and embedded in epon. Semithin sec-

Table 1. Light and immunofluorescence microscopy

Case No.	Mesangial enlargement ^{a)}	Specific fluorescence for ^{b)}	
		IgG	C3
1	++	+++	++
2	++	++	+
3	++	++	++
4	+	++	+
5	++	+++	++
6	++	+++	++
7	++	++	++
8	+++	+++	+++
9	++	+++	++
10	++	+++	+++
11	+++	+++	+++
12	+++	++	++
13	++	++	++
14	++	+++	++
15	+++	+++	+++
16	+++	++	++
17	++	++	+
18	++	++	+
19	++	+++	++
20	++	++	++
21	++	++	+
22	++	++	+
23	++	++	+
24	+	+	+
25	±	+	+
26	±	±	+
27	±	±	±
28	—	—	±
29	±	—	—
30	±	±	—
31	±	±	±
32	±	—	±

a) +++ severe, ++ moderate, + mild, ± slight or minor deviation from normal, — absence

b) +++ strong, ++ moderate, + mild, ± some fine fluorescence, — absence

tions were cut and stained with toluidine blue to select non-distorted glomeruli. Ultra-thin sections were cut, stained with uranyl acetate and lead citrate, and examined with a Hitachi H-300 electron microscope at 75 kV. At least two glomeruli per pig were examined.

Fig. 1. Global deposition of swine third complement component (C3) in the glomerulus. Case No. 19. ×58.

Fig. 2. Mesangial enlargement associated with electron-dense deposits. M=mesangial cells. Case No. 7. ×3,400.

Fig. 3. Large paramesangial deposits. Arrows indicate hemispherical deposits protruding into the urinary space. M=mesangial cell. Case No. 12. ×6,800.

Table 2 Dense deposits and spherical microparticles in electron microscopy

Case No.	Dense deposits ^{a)}				Spherical microparticles			
	PMes	Mes	SEn	SEp	Mes	SEp	SEn	IMem
1	++ ^{b)} (++) ^{c)}	++	+	-	++	++	-	-
2	++(++)	+	-	-	-	-	-	-
3	++(+)	+	+	-	-	-	-	-
4	++(+)	++	-	-	-	+	-	+
5	++(++)	+++	+	-	++	++	-	-
6	++(+)	+++	+	-	++	++	-	-
7	+++ (+++)	++	+	-	-	++	-	-
8	++(++)	++	++	++	+	+	-	-
9	++(+)	++	-	-	-	-	-	-
10	+++ (+)	+++	+	-	-	-	-	-
11	+++ (++)	+	+	-	-	-	-	-
12	+++ (+++)	+	-	-	-	-	-	-
13	+++ (+++)	+++	-	-	++	-	-	-
14	+++ (+++)	++	++	-	-	-	-	-
15	+++ (++)	++	-	-	+	-	-	-
16	+++ (++)	++	-	-	-	-	-	-
17	++(+)	+	-	-	+	++	+	+
18	++(+)	++	++	-	-	++	-	-
19	++(+)	++	-	-	-	++	-	++
20	++(+)	++	+	-	-	-	-	-
21	++(+)	+	+	-	+	+	+	-
22	++(++)	++	++	-	+	+	-	-
23	+++ (+)	+	-	-	-	+	-	-
24	-(-)	+	-	-	++	++	-	-
25	-(-)	+	-	-	-	+	-	-
26	++(+)	+	-	-	-	-	-	-
27	-(-)	-	-	-	+	-	-	-
28	-(-)	-	-	-	-	-	-	-
29	+(-)	+	-	-	-	+	-	-
30	-(-)	+	-	-	-	+	-	+
31	-(-)	+	-	-	-	-	-	-
32	-(-)	+	-	-	+	+	-	+

a) PMes: paramesangial deposits; Mes: mesangial deposits;
SEn: subendothelial deposits; SEp: subepithelial deposits;
IMem: intramembranous deposits.

b) Intensity of deposition. -: not detected, +: mild, ++: moderate, +++: severe.

c) Hemispherical large deposits in the parenthesis.

RESULTS

As shown in Table 1, 24 of the 32 pigs (Cases Nos. 1 to 24) showed diffuse global mesangial enlargement with definite IgG and C3 deposition of various intensities (Fig. 1). The other eight pigs (Cases Nos. 25 to 32) showed slight or no changes in the glomeruli with faint or no IgG or C3

deposition. Mesangial enlargement was due to increased mesangial matrix, hypercellularity and characteristic deposition of hyaline materials. In most cases, IgG and C3 were present in the mesangial area, but in one pig (Case No. 8) these were located also on capillary walls in granular appearance.

The major abnormality in electron mic-

Fig. 4. Large subendothelial deposit (arrow). Ep=epithelial cell. En=endothelial cell. Case No. 1. $\times 5,100$.

Fig. 5. Subendothelial deposits (arrows) associated with thin and irregular basement membrane-like structure. Ep=epithelial cell. Case No. 8. $\times 12,000$.

Fig. 6. Many subepithelial deposits with membranous transformation. Case No. 8. $\times 8,400$.

roscopy was the electron-dense deposits in the glomeruli (Fig. 2). These deposits were located in subendothelial, subepithelial and most frequently mesangial areas (Table 2). Those in the mesangium were designated as mesangial deposits and paramesangial ones according to their location. Paramesangial deposits were located just beneath GBM and frequently showed large hemispherical appearance (Fig. 3). Mesangial deposits in the mesangial matrix were small, and some of them were fused with paramesangial deposits.

Subendothelial deposits were present in lamina rara interna and encountered less frequently than those in the mesangial area. Most subendothelial deposits were small, but large ones were rarely seen in a few pigs (Fig. 4). Thin basement membrane-like structure was seen between those deposits and the endothelium (Fig. 5).

Subepithelial deposits in lamina rara ex-

Fig. 7. Clusters of SMP in the subepithelial areas with irregularity of GBM. A few SMP in the lamina densa (arrows). Ep=epithelial cell. Case No. 17. $\times 17,000$.

Fig. 8. Clusters of SMP in the mesangium. M=mesangial cell. Case No. 17. $\times 18,000$.

terna were seen in only one pig (Case No. 8) (Fig. 6). Partial membranous transformation occurred frequently around those deposits.

The degree of deposition of electron-dense deposits generally coincided with those in light and immunofluorescence microscopy.

Clusters of SMP were observed in 20 of the 32 pigs. They were found in subepithelial (Fig. 7), intramembranous (Fig. 7), subendothelial and mesangial areas (Fig. 8). SMP were most frequently encountered in the subepithelial area forming occasionally large cluster and GBM showed frequently irregular thickening and/or thinning (Fig. 9). The incidence of SMP did not always coincide with the intensity in light and immunofluorescence microscopy.

No conspicuous changes of GBM were observed except the electron-dense deposits

and SMP.

Epithelial cells occasionally showed villous transformation. Partial foot process fusion was frequently observed, especially in the areas with electron-dense deposits or SMP.

Loss of fenestration of capillary endothelium frequently occurred in association with subendothelial deposits.

DISCUSSION

In this study, electron-dense deposits were present most frequently in the mesangial areas. The deposition concurred with that of changes in light and immunofluorescence microscopy. Consequently mesangial enlargement was most likely attributed to the electron-dense materials which might have been deposited by accumulation of immune complexes (IC).

In the present study, characteristic large

Fig. 9. Clusters of SMP with attenuation of lamina densa (arrow). Ep=epithelial cell. Case No. 1. $\times 17,000$.

paramesangial deposits were present in most pigs. The presence of hemispherical paramesangial deposits is one of the morphologic characters of IgA nephropathy [4, 6, 7, 14]. Some investigators reported that paramesangial deposits were usually present in younger patients with less extensive disease, but in old patients or in biopsies with advanced glomerular lesion the deposits were dispersed evenly throughout the excessive matrix [4]. The animals examined in this study were young and long-term follow-up study might clarify the fate of paramesangial deposits.

In chronic hog cholera, there were massive dense deposits in the mesangium, chiefly in the paramesangial areas, and prominent leukocytic infiltration in the glomerular capillary lumens [5]. Distribution and granular character of electron-dense deposits found in the disease were

very similar to those in this study. There was, however, no accumulation of blood-borne cells in the glomeruli in this study. The difference might have derived from the biological activity of immune deposits.

The mesangium regulates glomerular filtration and serves for disposal of macromolecular filtration residues [8]. Macromolecular aggregates and particulates in the glomerular capillary lumen may pass through the endothelial fenestrae into the subendothelial space (lamina rara interna) and then to the mesangium [11] or pass through the endothelial-mesangial interface into the mesangium [9]. Then, they may be eliminated from the mesangium in various ways; (1) retrograde diffusion into the efferent glomerular capillaries [10], (2) excretion across the mesangial-epithelial barrier at the mesangium into the urinary space [10, 12], (3) transmesangial migration and elimination at the juxta-glomerular apparatus [10-12], (4) phagocytosis and digestion [3, 12, 18]. Mesangial deposition of circulating macromolecules is dependent on many factors including characters of the macromolecules, mechanics of the glomerular microcirculation, interactions between the matrix and transient macromolecules, and the potential pathways for egress [8]. Paramesangial deposits found in this study might have been formed due to disturbance of the excretion route and/or continuous excessive entering of circulating IC as well as other serum proteins. Further-more, it should be possible that the structure or physiological status of the glomeruli of the pig may easily allow the macromolecule to enter and stay in the mesangial area followed by enlargement.

In addition to electron-dense deposits, clusters of SMP were frequently observed. Those SMP were found also in swine glomeruli in our previous study [16] suggesting a high incidence of these in swine. In man, SMP was seen in various forms of

renal disease and implicated as degenerated cytoplasm, changed dense deposits or altered GBM [1, 2]. In this study, the frequency of the SMP deposition did not correlate to the degree of the deposition of the immunoreactants or electron-dense deposits. The origin of SMP was not clarified but from their location they might be formed *in situ* chiefly in the degenerating process of GBM or epithelial cells. A report [20] on IgA nephropathy stated that the deposition of SMP was encountered in 59% of patients, frequently in association with the thinning or disruption of the GBM and that hematuria in some patients with IgA nephropathy might develop from the gap of GBM associated with SMP formation. In this study, the distribution of SMP was identical to those in IgA nephropathy, but no definite gap of GBM was seen. In the present study, GBM showed no conspicuous changes except the electron-dense deposits or SMP. The minor involvement of GBM was an essential difference from IgA nephropathy in which the GBM lesions occurred frequently and independently of electron-dense deposits [6].

Ultrastructural lesions in this study varied in intensity, but the character of lesions might essentially be the same. The pathogenesis of glomerular changes was not elucidated in this study, but there should be a common cause from the morphological uniformity.

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要 約

豚の腎糸球体病変に関する微細形態学的研究：代田欣二・野村靖夫（麻布大学獣医学部病理学教室）—— 約6か月齢の豚32例の腎糸球体における微細形態学的変化を検索した。このうち24例は、光顕的に瀰漫性広汎性にメサンギウム拡大を示し、IgGとC3の沈着を伴っていたが、他の8例では糸球体病変およびIgG、C3沈着が軽微か、あるいは認められなかった。主な微細形態学的変化は、メサンギウム領域、内皮下、上皮下におけるdense depositの沈着で、メサンギウムでは、paramesangial depositが特徴的であった。dense depositの沈着程度は、光顕・免疫蛍光法における変化のそれと相関していた。32例中20例にはspherical microparticle (SMP)の集塊があり、それらは上皮下に最も顕著で、メサンギウム内、基底膜内、内皮下にも認められた。SMPの出現頻度は、光顕、免疫蛍光法における変化、dense depositの沈着程度とは必ずしも相関しなかった。dense depositあるいはSMP沈着を伴う部位を除いては、糸球体基底膜に著変を認めなかった。